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RESEARCH PAPER

Effect of different smoking times and temperatures on polycyclic aromatic hydrocarbon concentrations in smoked sausages

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Abstract

Smoked pork and chicken sausages were prepared using three different time-temperature combinations; 60 °C for 4 hours, 75 °C for 3 hours, and 90 °C for 2 hours. The amount of polycyclic aromatic hydrocarbons (PAHs) present in these smoked products was estimated using liquid chromatography-mass spectrometry (LC-MS). The PAH compounds were identified using multiple reaction monitoring (MRM) and the retention time of that particular compound. The most potent carcinogenic PAH compound, benzo(a)pyrene (BaP), was not detected in any of the treatments analyzed. The pork and chicken sausages were found to contain the following PAH compounds: Flu, BaA, Chr, BbF, and BkF. Chrysene was found in all samples in the range from 27.38 to 45.08 µg/kg. It was observed that a higher smoking temperature (90 °C) resulted in more PAH compounds in the sausages. Furthermore, the chicken sausages were found to have higher concentrations of PAHs, particularly Chr and BaA, than the pork sausages.

Keywords

Liquid Chromatography-Mass Spectrometry, Polycyclic Aromatic Hydrocarbons, Smoked sausages, Smoking parameters

Introduction

Smoking meat products has historically been practiced primarily for its ability to extend the shelf-life, and impart a desired color, flavor, and aroma to smoked foods (Lingbeck et al. 2014). The shelf-life extension is due to the combined antimicrobial and antioxidant effects of formaldehyde, carboxylic acids, and phenols in the smoke (Kim et al. 2014). However, thermal decomposition of wood in a certain temperature range (500–900 °C) can lead to the formation of harmful compounds, including polycyclic aromatic hydrocarbons (PAHs), which are known to be harmful to human health (Bartle et al. 1991). It is important to be aware of the potential health risks associated with the production and consumption of smoked meat products.

PAHs are a series of organic non-polar compounds that contain two or more fused aromatic rings (Danyi et al. 2009; Badry 2010) and have lipophilic, semi-volatile, and persistent properties (Plaza et al. 2010; Palm et al. 2011). The Environmental Protection Agency (EPA) has listed the sixteen PAHs, namely naphthalene (NAP), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLU), phenanthracene (PHEN), anthracene (ANTH), fluoranthene (FLTH), pyrene (PYR), benzo[a]anthracene (B[a]A), chrysene (CHR), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo [a]pyrene (B[a]P), benzo[g,h,i]perylene (B[ghi]P), indeno[1,2,3-c,d]pyrene (IND) and dibenz[a, h] anthracene (D[ah]A) as high-priority pollutant. The International Agency for Research on Cancer (IARC) (2010) has classified benzo[a]pyrene

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(BaP) in Group 1, i.e. "carcinogenic to humans", and the other compounds either as "probably carcinogenic", i.e. Group 2A, or classified Group 2B, i.e. "a possible carcinogen". According to the European Union (2011), the maximum limit for benzo[a]pyrene (BaP) in smoked meat and fish products is 5 µg/kg and for the total content of the four PAH compounds, namely, benzo[a]pyrene (BaP), chrysene (CHR), benzo[a]anthracene (BaA) and benzo[b]fluoranthene (BbF) (PAH4) as 30 µg/kg. PAHs are said to contribute around 1 to 20% of the total carcinogenic effects of smoked products (EU 2011).

The thermal treatment of food, for example, barbequing and grilling of meat products, produces carcinogenic compounds (Moazzen et al. 2013). PAHs in food are formed by the pyrolysis of nutrients present in the food and by the deposition of smoke resulting from incomplete combustion of thermal substances (Hamidi et al. 2016).

The amount and concentration of PAHs to which one is exposed, the route and duration of exposure, and their relative toxicity all play an important role in their effects on human health. Numerous PAHs are typically considered carcinogens, mutagens, and teratogens and pose a serious threat to human health and well-being. According to epidemiological research from the World Health Organization (2021), PAHs are associated with reduced lung function, asthma attacks, and a higher incidence of obstructive pulmonary disease and cardiovascular disease. Furthermore, little epidemiological evidence suggests that it has negative effects on children's cognitive or behavioral functions (WHO 2021). Viegas et al. (2012) stated that the PAH content in smoked meat and fish significantly increases the risk of stomach cancer. Additionally, higher prenatal PAH exposure is associated with lower IQ at age three, increased behavioral problems at ages six and eight, and childhood asthma. The umbilical cord blood of exposed babies showed DNA damage that has been linked to cancer (Edwards et al. 2010; Shafy and Mansour 2016). IPCS (2010) found that anthracene and benzo(a)pyrene are skin sensitizing, i.e. they can cause an allergic reaction in both humans and animals. PAHs such as naphthalene, benzo(a)pyrene, anthracene, and a mixture of various other PAHs can even cause skin irritation and inflammation.

The present study investigated how different time-temperature combinations of smoking, affect the formation of 9 PAHs, viz., fluoranthene, chrysene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, indeno(1,2,3-cd) pyrene, benzo(a)pyrene, benzo(ghi)perylene and dibenzo(a,h)anthracene, in smoked pork and chicken sausages. The results of the present study are intended to serve as valuable data on the safety of smoked meat products and the practices to be followed while smoking foods.

Materials and methods

The study was accomplished at the College of Veterinary Science, Assam Agricultural University, Khanapara, All India Coordinated Research Project on Post Harvest Engineering and Technology (AICRP on PHET), Khanapara, Guwahati (Assam) and the ICAR - National Meat Research Institute (Formerly NRC on Meat), Chengicherla, Hyderabad (India).

Source of raw material

The pork loin chops and lard, along with chicken breast and skin, were obtained from the sales booth on the college campus. The meat was cut into chunks of 2–3 cm and kept at a temperature of -18 ° C until further processing. Best-quality spices (namely Jeera, Dhania, Black Pepper, and Kashmiri red chilies, Make: Everest spices), binder, and condiments (Onion, Ginger, and Garlic powder, Make: Keya Foods) were obtained from the Beltola market, Guwahati. Quechers Disque AOAC salt and solid phase extraction (SPE) HLC 6cc Cartridges (Waters India) were used for the extraction of the PAH compounds. The standards for the 9 PAH that were employed in the study were purchased from Sigma Aldrich (USA).

Preparation of the sausage

Frozen lean meat and fat were thawed overnight at 4 ± 1 °C. The thawed meat chunks were then minced using a mechanical meat mincer (Make: Sun Labz, Model: TC 12) with a sieve diameter of 4 mm. Curing agent was added to the minced meat according to the amount shown in Table: 1 and left to rest for 24 hours to achieve proper curing. The cured meat was then placed in a bowl chopper where other ingredients such as fat, spices, seasonings, and non-meat ingredients were added to form an emulsion. The emulsion was then filled into cellulose casings and the raw sausages were cooked in a cooking vat at a temperature of 85 °C for 45 minutes. Immediately after cooking, the sausages were placed in chilled water (4 ± 1 °C) to neutralize the latent heat, prevent overcooking, provide thermal shock to any microorganisms present, and prevent further cooking.

Table 1. Formulation for sausage preparation.

Ingredients	Proportion (%)
Meat (Chicken/ Pork)	80
Fat (Lard/Skin)	20
Total	**100**
Spices	1.5
Condiments	3
Binder (Corn flour)	3
Ice cubes	10
Sugar	0.5
Salt	2.3
Sodium nitrite	0.15
Sodium triphosphate (STPP)	0.5
Ascorbic acid	0.2

Smoking of the sausages

The cooked pork and chicken sausages were placed in a smoke oven (Make: Henan Xuanhua) and were smoked with teakwood sawdust for various time-temperature combinations. The three combinations examined were 60 °C for 4 hours, 75 °C for 3 hours, and 90 °C for 2 hours. After smoking, the sausages were cooled, peeled, and packaged in vacuum-sealed packages. They were then stored at −18 °C until analysis. In total, there were six treatments labeled P60, C60, P75, C75, P90, and C90. Here C stands for chicken and P for pork.

Extraction method of PAHs for LC-MS

The AOAC (2012) method was used for the extraction of PAHs and clean-up of the smoked sausages. After homogenizing the sausages, 15 grams of smoked pork and chicken sausage were taken in a centrifuge tube, and 15 ml of chilled acetonitrile (ACN) was added and shaken for 1 minute before vortexing it for 10 minutes. Then Quechers Disque AOAC salt comprising 1.5 g sodium acetate and 6 g magnesium sulphate (Waters India) was added and shaken vigorously for 2 minutes. After centrifuging the mixture at 40 °C for 10 minutes at 5000 rpm, 3 ml of supernatant was collected and passed through solid phase extraction (SPE) using an Oasis prime HLC 6cc Cartridge (Waters, India). An initial 1 ml of the eluent was discarded, and the remaining 2 ml was collected in glass tubes, which were then transferred to glass vials for injecting into LC-MS.

Quantification of PAHs by LC-MS

The extract was analyzed on Acquity UPLC coupled with a Mass spectrometer as per the method of Wu et al. 2017. The LC system was connected with "PAH C-18; 150*2.1 mm, 5 µm column. Helium was used as the carrier gas. The column was maintained at a constant flow rate of 0.6 ml/min, and 10µl of aliquot was injected. The voltage in the capillary was set to 3.13 KV, and the voltage in the cone was set to 25V. The desolvation temperature was set at 300 °C, the desolvation flow was set at 650L/hours, and the collision energy was set at 14V. The inlet temperature was maintained at 275 °C. The column temperature program for PAH analysis was initially set at 70% ACN at 0.01 min and then increased to 80% ACN at 3 min and 98% ACN at 8 min, which is maintained constant at 9 min and decreased to 70% ACN at 9.5 min. A blank sample was run for 12 minutes, and matrix-matched standards were employed. Individual PAH standards were used at a concentration ranging from 5 to 100 µg/kg. The total run time was set for 12 min. The identification of various PAHs viz., Fluoranthene, Chrysene, Benzo(a)anthracene, Benzo(k)fluoranthene, Benzo(a)pyrene, Benzo(b)fluoranthene, Indeno (1,2,3-cd) pyrene, Benzo (g,h,i) perylene, dibenzo (a,h) anthracene was accomplished utilizing a comparison of the retention times and mass spectra of unknown peaks to those of the reference standards. After LC-MS analysis, the standard curve of each PAH was obtained, and correlation coefficients were determined with an Excel software system.

All standard compounds, each at different concentrations, were injected into the system. The linearity was drawn, and the correlation coefficient (r^2) was also determined, which lay within the range of 0.095 to 1. Limits of detection (LODs) and quantification (LOQs) were calculated based on the signal-to-noise ratio of equal to 3 and 10, respectively. The LODs and LOQs were found to be 5 µg/kg and 10 µg/kg, respectively. The retention time of the 9 PAH compounds is given in Table 2. The chromatographs of the PAH compounds found in the samples, viz., Fluoranthene, Benzo (A) Anthracene, and Chrysene are presented in Fig. 1.

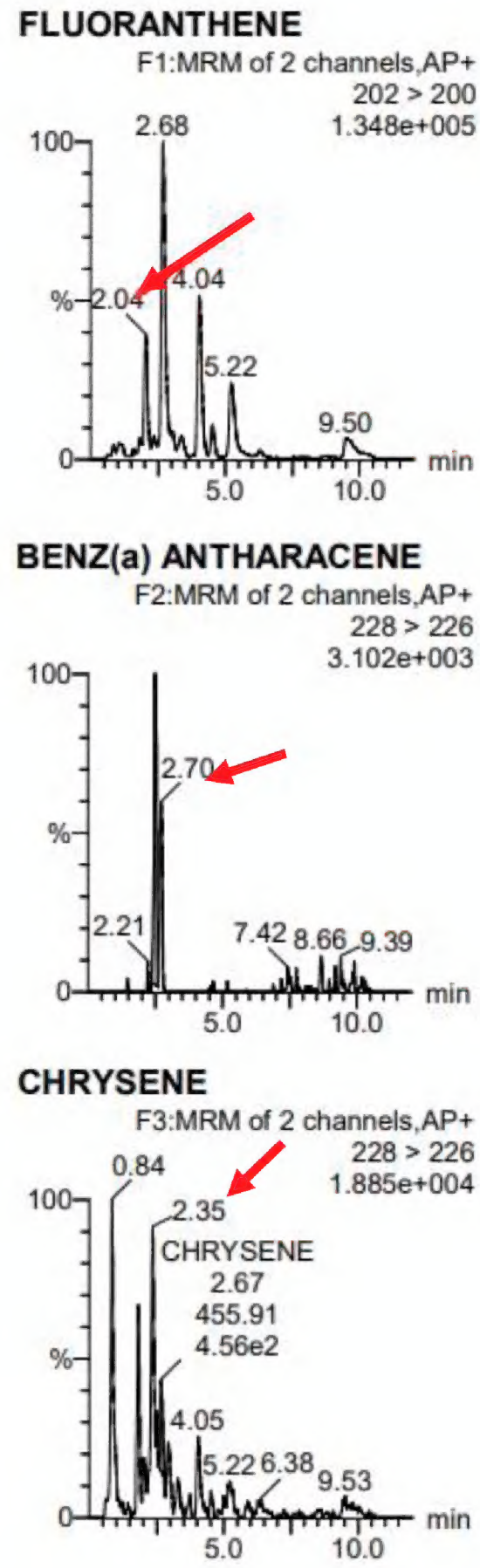

Figure 1. Chromatographs of Flouranthene, Benzo (A) Anthracene and Chrysene of smoked pork and chicken sausages using different smoking time and temperature regimes.

Table 2. Retention time of the studied Polycyclic Aromatic Hydrocarbons (PAH) compounds.

Compound name	Retention Time(min)
Flouranthene	1.707
Benzo (A) Anthracene	2.52
Chrysene	2.73
Benzo (A) Pyrene	3.47
Benzo (B) Fluoranthene	3.904
Benzo (K) Fluoranthene	4.39
Indeno (1,2,3- CD) Pyrene	5.86
Benzo (G,H,I) Perylene	5.53
Dibenz (A, H) Anthracene	5.179

Statistical analysis

The data of the studied PAH compounds in smoked pork and chicken sausages were analyzed statistically using the one-way ANOVA tool of the data analysis software SPSS (16).

Results and discussion

The mean ± SE values for PAH compounds, Fluoranthene, Benz(a)anthracene, and Chrysene that were detected in the treatments of the smoked pork and chicken sausages with different time-temperature combinations of smoking are presented in Table 3.

The treated pork and chicken sausages did not show the presence of the most potent carcinogen, Benzo (a) pyrene. The samples were also absent of Benzo (b) fluoranthene, Benzo (k) fluoranthene, Indeno (123cd) pyrene, Benz (ghi) perylene, and Dibenz (a,h) anthracene. Benzo (k) fluoranthene and Benzo (b) fluoranthene were below the limit of quantification (BLQ), i.e., below 10µg/kg for C60, C75, and C90.

Table 3. Concentration of Polycyclic Aromatic Hydrocarbons (PAHs) (µg/kg) of smoked pork and chicken sausage prepared with different time-temperature combinations.

TREATMENTS	CONCENTRATION OF PAHs				
	Flu	BaA	Chr	BbF	BkF
P60	ND	ND	27.38 ± 1.27^{d}	ND	ND
C60	ND	ND	35.02 ± 0.87^{c}	BLQ	ND
P75	ND	ND	42.58 ± 0.47^{b}	ND	BLQ
C75	ND	ND	45.08 ± 0.39^{a}	BLQ	BLQ
P90	11.18 ± 0.15	26.6 ± 0.32^{b}	40.82 ± 0.32^{b}	ND	ND
C90	ND	47.48 ± 0.52^{a}	40.78 ± 0.32^{b}	BLQ	BLQ

ND - Not detected, BLQ - Below the level of quantification. Flu - Fluoranthene, BaA - Benz (a)anthracene, Chr - Chrysene, BbF - Benzo (b) fluoranthene, BkF - Benzo (k) fluoranthene;
n = 5, Means with superscript bearing different alphabet (small) column-wise differ significantly ($P < 0.05$).

The levels of chrysene were found to be significantly ($p < 0.05$) higher in the treatment C75 group samples, and the lowest in treatment P60. The BaA was observed only in the treatment P90 and C90 groups. Flu compound was not detected in any of the treatment groups except for the P90 group. This might be due to the higher temperature used for the smoking process. It was also noticed that the smoked chicken sausages had significantly ($p < 0.05$) higher PAH concentration than smoked pork sausages at 60 °C for 4 hours and 75 °C for 3 hours. This might be due to the level of unsaturated fatty acid, which may affect the heterogeneity and concentration of PAHs formed in smoke (Chen and Chen 2001). However, the difference at 90 °C for 2 hours, was insignificant.

The results recorded in the present study were comparable to those of Racovita et al. (2020) who studied the influence of smoking temperature, smoking time, and type of wood sawdust, on PAH levels of pork sausages, and observed that PAH concentrations increased continuously both with higher temperatures (55–95 °C) and with longer smoking periods (2–9 h). The level of benzo[a]pyrene tended to plateau after 6 h. Wood such as plum, alder, and birch wood yielded higher PAH concentrations in their study.

Similarly, Alsadat et al. (2021) reported a positive correlation between the lignin content of smoke generation sources and the PAHs level of smoke and smoked sausages. Also, the total PAH contents in both smoke and smoked sausages increased significantly as smoking duration increased from 2 to 4 h. Kafouris et al. (2020) reported that 12% of the smoked meat products (19 out of 159 samples) in Cyprus exceeded the MLs of the EU legislation applied for each foodstuff. However, the smoking was carried out for days and months and the temperature did not surpass 28 °C. Tiwo et al. (2019) reported an increase in lipid content was found to increase the PAH content in smoked fish, attributed to its lipophilic activity.

The presence of BaA and the decrease in the Chr content in treatment P90 and C90, having a temperature-time of 90 °C for 2 hours might be due to vaporization or conversion of the compound to some other PAH derivatives (Chen and Chen 2001).

Conclusion

The present study reports that a time-temperature combination is crucial for the production of polycyclic aromatic hydrocarbons. It is observed that pork and chicken sausages cooked at 90 °C for two hours contain the highest amounts of PAH compounds. Among the estimated PAH compounds, chrysene dominated in all treatments. Certain PAH compounds could not be detected in the present study, which may be due to the lower time-temperature combination used. According to the current study, the temperature-time combination of 60 °C for 4 hours was found to be suitable concerning lesser PAH compound formation and also for the development of all smoked features. Unrestricted smoking of meat products can have harmful health effects and thus, hurdle technology should be used to reduce the concentration of the PAH compound in the product. Therefore, it is the need of the hour to conduct further studies applying various hurdle technologies to reduce the concentration of the PAH compound and set standards for smoking various meat products.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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